Citation:

Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *J Nutr* 2004;134(11): 2991-2997.

PubMed ID: 15514264

Study Design:

Randomized Crossover Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To evaluate the effects of alpha-linolenic acid (ALA) on multiple cardiovascular disease (CVD) risk factors.

Inclusion Criteria:

- Moderate hypercholesterolemia: serum total cholesterol (TC) = 5.17- 6.21 mmol/L; LDL cholesterol (LDL-C) = 40th - 90th percentile
- Overweight/obesity class 1: BMI = 25-35 kg/m^2
- Not taking lipid-lowering anti-inflammatory medications and/or dietary supplements
- Nonsmokers
- No documented atherosclerotic disease, inflammatory disease, diabetes mellitus, uncontrolled hypertension ($\geq$ 140/90 mm Hg) or other systemic diseases
- Postmenopausal, and not receiving hormone replacement therapy

Exclusion Criteria:

Not specified.

Description of Study Protocol:

Recruitment : Recruitment methods not specified.

Design : Randomized, controlled, crossover trial

Blinding used (if applicable): not mentioned

Intervention (if applicable)

- 3 experimental diets [fat: 35% energy; carbohydrate: 50% energy; protein 15% energy; cholesterol: 300 mg/d]
  - AAD - average American diet (control)
  - ALA diet - high in PUFA and ALA
  - LA diet - high in PUFA and linoleic acid

Percent energy fatty acids in three test diets

	AAD	LA	ALA
SFA	13%	8%	8%
MUFA	13%	13%	13%
PUFA		16-17%	16-17%
LA	9%	12.6%	10.5%
ALA		3.6%	6.5%
LA:ALA	10:1	4:1	2:1

- Diet was developed at 8 energy levels (7524 kJ to 16,302 kJ)
- 6 day cycle menu for each energy level (Nutritionist V database)
- Each diet period was 6 weeks with ≤ 3 week break between diet periods to improve diet compliance

Statistical Analysis - performed using SAS v8.2

- Differences among 3 experimental diets: ANOVA
- Effects of diet, order and interactive effect on outcomes: mixed procedure (PROC MIXED); significant effects tested using Tukey's least significant difference test
- Associations between lipid/lipoprotein variables and novel CVD risk factors - Pearson correlation analysis
- Data for men and women were pooled (except in ANOVA)
- For variables with nonnormal distributions, medians and geometric means $\pm$ SEM are reported and statistical analysis were conducted after a logarithmic (base 10) transformation

Data Collection Summary:

Timing of Measurements

Clinical and biochemical analyses completed at baseline and at the end of each diet period.

Dependent Variables

- Serum C-reactive protein (CRP): ELISA assay
- Serum intercellular cell adhesion molecule-1(sICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin: quantitative sandwich enzyme immunoassay kits (R&D Systems)
- Serum total cholesterol (TC), HDL cholesterol (HDL-C) and triglycerides (TG): enzymatic method as described by Yu-Poth et. al.
- LDL-cholesterol (LDL-C): Friedewald's equation: LDL-C - TC - (HDL-C + TG/5)
- Apolipoprotein (apo) A1 and apo B - rate immunonephelometry (Beckman Assay)

- Serum fatty acid profile: GC

Independent Variables

- 2 experimental diets and control diet

Control Variables

Description of Actual Data Sample:

Initial N: 23; male: N=20; female: N=3

Attrition (final N): 23

Age: (mean ± SEM): total N: 49.8 ± 1.6; males: 48.6 ± 1.6; females: 58.3 ± 2.7 years

Ethnicity: not specified

Other relevant demographics: none specified

Anthropometrics

- Body weight (kg)(mean ± SEM): All: 86.7 ± 2.8; males: 88.5 ± 2.8; females: 74.9 ± 8.3
- BMI (kg/m^2) (mean ± SEM): All: 28.1 ± 0.7; males: 28.0 ± 0.7; females: 28.5 ± 2.4

Location: Pennsylvania State University, University Park, PA

Summary of Results:

Key Findings

- The ALA diet decreased C-reactive protein (P < 0.01), whereas the LA diet tended to decrease CRP (P = 0.08).
- Although both high-PUFA diets similarly decreased intercellular cell adhesion molecule-1 vs the average American diet, (-19.1% by the ALA diet, -11.0% by the LA diet, both P < 0.01), the ALA diet decreased vascular cell adhesion molecule-1 (-15.6% vs -3.1%, P < 0.01) and E-selectin (-14.6% vs -8.1%, P < 0.01) more than the LA diet.
- Changes in CRP and VCAM-1 were inversely associated with changes in serum EPA (r = -0.496, P = 0.016 and r = -0.418, P = 0.047) or EPA + DHA (r = -0.409, P = 0.053 and r = -0.357, P = 0.091) after subjects consumed the ALA diet.
- Both high-PUFA diets decreased serum total cholesterol, LDL cholesterol and triglycerides similarly (P < 0.05).
- The ALA diet decreased HDL cholesterol and apolipoprotein A1 compared with the average American diet (P < 0.05).

Fatty acid composition of serum lipids

- Changes in serum fatty acid profiles reflected the fatty acid composition of the 3 test diets
- Relative to AAD, serum total (n-6) PUFA (and LA) was higher after subjects consumed the LA diet (P<0.05)
- Serum total (n-3) PUFA [i.e. ALA, eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA), but not DHA], was higher after subjects consumed both the LA and ALA diets (P <

0.05)
- Serum total (n-6) PUFA was lower [both in LA and arachidonic acid (AA) and serum total (n-3) PUFA was higher (in ALA, EPA, and DPA) when subjects consumed the ALA diet compared with the LA diet
- Serum ratios of LA:ALA and (n-6):(n-3) were lower when subjects consumed the LA and ALA diets vs. the AAD ($P < 0.05$)

Serum CRP concentrations

- Compared to AAD (median serum CRP =1.51 mg/L), CRP levels
  - decreased 75% when subjects consumed the ALA diet (median serum CRP = 0.37 mg/L) ($P < 0.01$)
  - decreased 45% when subjects consumed the LA diet (median serum CRP = 0.83 mg/L) ($P = 0.08$)
  - No difference in changes with ALA and LA diets
- Lower CRP levels may be associated with lower TG levels and TC:HDL-C ratios with the ALA diet.
  - Correlation between CRP and TG: $r = 0.504$, $P < 0.05$
  - Correlation between CRP and TC:HDL-C: $r = 0.482$, $P < 0.05$
  - These relations were not observed when subjects consumed AAD or LA diets
- With LA diet:
  - Correlations between CRP and HDL-C: $r = -0.360$, $P < 0.05$
- With AAD diet
  - correlation between CRP and HDL-C: $r = -0.344$, $P < 0.05$
  - subjects with low (< 2 mg/L) CRP on AAD had greater reduction in LDL-C levels with LA and ALA diets vs AAD (-0.51 + 0.06 mmol/L) than those with high (≥ 2 mg/L) CRP levels (-0.36 + 0.06 mmol/L), $P = 0.068$

Serum cell adhesion molecule concentrations

- LA and ALA diets significantly decreased serum ICAM-1 and E-selectin compared with AAD ($P < 0.01$)
- ALA reduced VCAM-1 levels compared with AAD ($P < 0.01$)
  - VCAM-1 levels did not differ after LA and AAD
- ALA diet resulted in greater decreases in both VCAM-1 (12.9% decrease, $P < 0.01$) and E-selectin (7.2% decrease, $P < 0.01$) levels compared with the LA diet
- Dietary ALA may decrease serum ICAM-1 and lipid ratios in a parallel manner:
  - correlation between ICAM-1 and TC:HDL-C: $r = 0.444$, $P < 0.05$
  - correlation between ICAM-1 and LDL-C:HDL-C: $r = 0.464$, $P < 0.05$

Changes in serum CRP and VCAM-1 predicted by changes in serum (n-3) fatty acids

- Serum EPA may play a role in regulating CRP and VCAM-1 levels.
  - With ALA diet:
    - changes in serum CRP ($r = -0.496$, $P = 0.016$) and VCAM-1 ($r = -0.418$, $P = 0.047$) were inversely associated with changes in serum EPA (vs. AAD)
    - trend for inverse correlations also existed between changes in CRP and VCAM-1 and changes in serum EPA+DPA, and between changes in CRP and changes in serum ALA+EPA+DPA ($P < 0.1$)
  - With LA diet:
  - changes in CRP and changes in serum EPA: $r = -0.353$, $P = 0.099$) and EPA+DPA: $r = -0.386$, $P = 0.069$

Serum lipid and lipoprotein concentrations

- Lipid lowering effects of ALA and LA diets were comparable compared to AAD (see table below)
- ALA significantly decreased HDL-C ($P < 0.05$) and apo A1 ($P < 0.05$) compared with AAD, but there were no differences in HDL-C and Apo A1 levels when subjects consumed the LA and ALA diets, and both reduced TC:HDL-C ratios similarly

Other Findings

Percent reduction in serum lipids with LA and ALA diets compared with AAD diet

	LA	ALA
Serum TC	10.9%	10.8%
LDL-C	12.3%	11.0%
TG	18.4%	18.4%
apo B	9.4%	9.7%

Serum lipid, lipoprotein, and apolipoprotein concentrations in subjects when they consumed the AAD, LA diet, and ALA diet for 6 weeks (mean $\pm$ SEM)

	AAD	LA diet	ALA diet
TC (mmol/L)	5.59 ± 0.16^b	4.98 ± 0.13^a	4.99 ± 0.14^a
LDL-C (mmol/L)	3.74 ± 0.14^b	3.28 ± 0.12^a	3.33 ± 0.11^a
HDL-C (mmol/L)	1.18 ± 0.06^b	1.15 ± 0.06^{ab}	1.11 ± 0.05^a
TG (mmol/L)	1.47 ± 0.13^b	1.20 ± 0.11^a	1.20 ± 0.11^a
TC:HDL-C	4.90 ± 0.18^b	4.52 ± 0.18^a	4.65 ± 0.19^a
Apo A1 (g/L)	1.51 ± 0.04^b	1.45 ± 0.05^{ab}	1.43 ± 0.04^a

Means in a row with superscripts without a common letter differ, $P < 0.05$

Author Conclusion:

A diet high in PUFA, especially ALA, elicits cardioprotective effects by decreasing lipid and lipoprotein levels and by eliciting vascular anti-inflammatory effects.

Reviewer Comments:

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	???
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	???
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes

3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?		Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)		Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?		N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)		N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?		N/A
4.	**Was method of handling withdrawals described?**		Yes
4.1.	Were follow-up methods described and the same for all groups?		Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)		Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?		Yes
4.4.	Were reasons for withdrawals similar across groups?		N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?		N/A
5.	**Was blinding used to prevent introduction of bias?**		Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?		???
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)		Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?		N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?		N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?		N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**		Yes

6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes

8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A

9. Are conclusions supported by results with biases and limitations taken into consideration?   Yes

9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes

10. Is bias due to study's funding or sponsorship unlikely?   ???

10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	???